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ABSTRACT

The present invention relates to the use of aliphatic carbocyclic compounds that have pendant hydroxy groups for incorporating water-insoluble molecules as hydrophilic components of liposomal drug delivery systems for human or animal treatment.

In particular this patent describes a liposomal drug delivery system wherein the hydrophilic component contains in part a 17-ketosteroids along with aliphatic carbocyclic compounds that have pendant hydroxy groups, such as cyclodextrin (CD), and the likes, as well as mixtures thereof. In particular the 17-ketosteroids is Dehydroepiandrosterone(DHEA) and the cyclodextrin is hydroxypropyl-beta-cyclodextrin. The cyclodextrin is used with the 17-ketosteroid to give it hydrophilic characteristics.

The present invention also relates to the use of certain 17-ketosteroids as hydrophobic components of liposomal drug delivery systems. As hydrophobic elements, of liposomes, the 17-ketosteroids have been discovered to have beneficial rapid drug delivery characteristics hitherto unknown.

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APPLICATION No.....

PATENT APPLICATION

INVENTOR: PATRICK T. PRENDERGAST

TITLE: LIPOSOMAL FOR CONTAINING ALIPHATIC CARBOCYCLIC COMPOUNDS WITH PENDANT HYDROXY GROUPS

Patrick T. Prendergast

BACKGROUND TO THE INVENTION

Any prescribed drug dose is the result of compromise. On the one hand, all drugs are potentially poisonous, suggesting that the least possible amount should be administered. On the other hand, drugs become diluted in the blood and large amounts are degraded, taken up by healthy tissues or excreted without ever reaching the site of disease. Such wastage increases the need for high doses. Physicians balance these opposing pressures by prescribing doses they think will be high enough to control the patient's problem but low enough to avoid causing unacceptable damage to healthy tissues.

To reduce the risk and inefficiency associated with such guesswork, many laboratories are now developing drug-delivery systems that alter the pathways by which drugs travel through the body. The goal is to deliver the needed dose of medicine to diseased tissues but to bypass healthy ones, thereby improving the drug's ratio of effectiveness to toxicity. One highly promising approach to achieving this goal is the loading of medication into liposomes, which are microscopic sacs made of the very phospholipids that constitute cell membranes.

Liposomes can be filled with a variety of medications and, because of their similarity to cell membranes, are not toxic. They also protect their loads from being diluted or degraded in the blood. As a result, when the liposomes reach diseased tissues, they deliver concentrated doses of medication. Liposomes containing a variety of drugs have been shown in many animal studies, and in some clinical tests, to be more effective and less toxic than free drugs.

Alec D. Bangham of the Agricultural Research Council's Institute of Animal Physiology in Cambridge, England, inadvertently produced the first liposomes in 1961 while evaluating the effect of phospholipids on blood clotting. When Bangham put water in a flask containing a phospholipid film, the water forced the molecules to arrange themselves into what he later discovered were microscopic closed vesicles composed of the bilayered (two-molecule-thick) phospholipid membrane surrounding water entrapped from the environment.

Phospholipids form closed, fluid-filled spheres when they are mixed with water in part because the phospholipid molecules are amphipathic: they have a hydrophobic (water-insoluble) tail and a hydrophilic (water soluble), or "polar", head. Two fatty acid

chains, each containing from 10 to 24 carbon atoms make up the hydrophobic tail of most naturally occurring phospholipid molecules. Phosphoric acid bound to any of several water-soluble molecules composes the hydrophilic head. When a high enough concentration of phospholipids is mixed with water, the hydrophobic tails spontaneously herd together to exclude water, whereas the hydrophilic heads bind to water.

The resultant bilayer in which the fatty acid tails point into the membrane's interior and the polar head groups point outward is called a liposome. The polar groups at one surface of the membrane point towards the liposome's interior and those at the other surface point toward the external environment. It is this remarkable reactivity of phospholipids to water that enables workers to load medications into liposomes. As a liposome forms, any water-soluble molecules that have been added to the water are incorporated into the aqueous spaces in the interior of the spheres, whereas any lipid-soluble molecules added to the solvent during vesicle formation are incorporated into the lipid bilayer.

Liposomes employed for drug delivery typically range in diameter from 250 angstrom units to

several micrometers (the diameter of a red blood cells is roughly 10 micrometers) and are usually suspended in a solution. They have two standard forms: "onion-skinned" multilamellar vesicles (MLV's), made up of several lipid bilayers separated by fluid, and unilamellar vesicles, consisting of a single bilayer surrounding an entirely fluid core. The unilamellar vesicles are typically characterised as being small (SUV's) or large (LUV's).

Under appropriate circumstances liposomes can be absorbed into almost any cell type. Once they have been adsorbed the spheres may be endocytosed, or swallowed up, by some cells. Adsorbed liposomes can also exchange lipids with cell membranes and may at times be able to fuse with cells. When fusion takes place, the liposomal membrane is integrated into the cell membrane and the aqueous contents in the cell.

SUMMARY OF THE INVENTION

The present invention relates to the use of aliphatic carbocyclic compounds that have pendant hydroxy groups for incorporating water-insoluble molecules as hydrophilic components of liposomal drug delivery systems for human or animal treatment.

In particular this patent describes a liposomal drug delivery system wherein the hydrophilic component contains in part a 17-ketosteroid along with or combined with an aliphatic carbocyclic compounds that have pendant hydroxy groups, such as cyclodextrin (CD),and the likes, as well as mixtures thereof. In particular the 17-ketrosteroid is Dehydroepiandrosterone(DHEA) and the cyclodextrin is hydroxypropyl-beta-cyclodextrin. The cyclodextrin is used with the 17-ketosteroid to give hydrophilic characteristics. Additional therapeutic efficiency is obtained where DHEA is required as the active agent resulting from the fact that when DHEA is complexed with beta-cyclodextrin the compound is not readily transformed to DHEA-sulphate within the body.

The present invention also relates to the use of certain 17-ketosteroids as hydrophobic components of liposomal drug delivery systems. As hydrophobic

elements, of liposomes, the 17-ketosteroids have been discovered to have beneficial rapid drug delivery characteristics hitherto unknown. Liposomes having 17-ketosteroids as hydrophobic components are thus claimed to be ideal carriers for the delivery of any therapeutic agent and in particular toxic therapeutic agents wherein the therapeutic agent forms part or the total hydrophilic component of the liposome.

The preparation of suitable hydroxypropyl-beta cyclodextrins is described, inter alia, in International Journal of Pharmaceutics 29:73-82 (1986) and in Journal of Pharmaceutical Sciences 75 (6):571-572 (1986). Also known, and contemplated for the purposes of the present invention are the hydroxypropyl-beta-cyclodextrins that are polyesters of cyclodextrins and are obtained by condensation of an excess of hydroxypropylene oxide with beta-cyclodextrin as described in U.S. Pat. No. 3,459,731 to Gzamera et al. Historically, cyclodextrins (CDs) have been used extensively in the pharmaceutical industry in oral formulations, parenteral formulations and suppositories. In practical terms, CD complexes improve drug stability, enhance solubility, promote faster absorption, reduce local irritation and result in improved bio-availability.

This method of encapsulating hydrophobic molecules in the hydrophilic compartment of liposomes using cyclodextrins has definite advantages over the classical method of dissolving in organic solvents. Complexation is favoured in cold, concentrated CD solutions. Equilibrium is shifted in warm, dilute solutions and molecules of interest or guest molecules are released. Stability is conferred to the guest molecule by protecting it from degradation due to heat, sublimation, enzymatic sulphation, oxidation and/or light. Improved bio-availability of components that have been complexed also occurs because their homogeneous distribution is increased and their transfer into a molecular-dispersed state is facilitated upon release from the liposome. Many of the 17-ketosteroids function as hormones and include sex hormones or precursors thereof and hormones which control metabolism. Dehydroepiandrosterone (DHEA) is one such 17-ketosteroid which is a precursor of both androgens and estrogens and additionally has important metabolic effects. DHEA has been found to suppress some of the metabolic disorders and liver cirrhosis, and reduces pain in ischemic heart disease, especially in angina pectoris, by restricting tissue respiration. DHEA has been used in the treatment of menopause,

emotional instability, depression and stress. DHEA and related compounds are capable of reducing the colony forming ability of human peripheral blood mononuclear (PBM) cells infected with Epstein-Barr virus (a herpes virus) at concentrations of 10-100 uM (Carcinogenesis, Vol. 2, pp 883-886, 1981).

DHEA also inhibits complement activation and is therefore of value in the prophylaxis of Hereditary Angioneurotic Oedema (Hidvegi et al., Complement 1; 201, 1984). DHEA also prevents autoantibody formation in the murine model of Systemic Lupis Erythematosus (SLE) and many of the features of full-blown AIDS are considered to be similar to those of SLE (lucas et al., J. Clin. Invest., 75: 2091, 1985).

Recent studies in animals demonstrate that DHEA has beneficial effects in obesity and breast cancer. Schwartz Cancer Res. 39:1129 (1979); Schwartz Nutrition and Cancer, 3:46 (1981), DHEA also has been shown to have antihypercholesterolic effects in lowering lipid levels in rats. Ben-David et al., Proc. Soc. Exp. Biol. Med., 20 125:1136 (1967).

The importance of hypercholesterolemia, an elevated low-density lipoprotein (LDL) cholesterol level, as a major risk factor for the development of ischemic heart disease is widely accepted.

Barrett-Connors et al., New Engl. J. Med. 315:1519 (1986) showed that individuals with low circulating levels of DHEA-S die of heart disease at a higher rate than normal subjects. The oral administration of DHEA (1600 mg/day) reduces total serum cholesterol and LDL level by about 7.1 and 7.5 percent, respectively, in normal subjects.

The use of DHEA and other 17-ketosteroids as medication for the prophylaxis and therapy of a retrovirus infection or for complications arising therefrom, e.g., Acquired immune deficiency syndrome (AIDS) has been reported in SCRIP No. 1422, June 21, 1989, page 21 and in British Pat. Publication No, 2,204,237 by Colthurst, Ltd. Oral administrations of relatively large doses of 1 to 2 grams per day has been tested in AIDS patients and shown to improve their immune systems and lower viral HIV load. In such tests, DHEA was administered orally alone or in combination with immunomodulators. Liposomes carrying DHEA as a hydrophilic CD complex target HIV infected macrophages and Kuffer cells more directly and thus reduce the dosage required, this formulation prevent sulphation to inactivate DHEAS form in the plasma and deliver the required dosage to the infected target tissues.

Studies with Liposomal Dehydroepiandrosterone (DHEA) in Mice

Study 1: Fate of liposomal DHEA after i.v. injection into mice.

Liposomes composed of soy lecithin and incorporating DHEA (mixed with radiolabelled DHEA) were injected i.v. (0.25 ml containing 0.9 mg DHEA) into Balb/c inbred mice. Animals were bled from the tail vein at time intervals and killed at 24 hrs after injection. Blood plasma samples and tissues obtained at death were analysed for radioactivity (^3H). Results are shown in Tables 1 and 2.

Table 1: Clearance of liposomal DHEA from the circulation

Mouse	% of injected liposomal DHEA in total blood				
	2 min	30 min	2.5 hours	7 hours	24 hours
1	9.2	3.1	1.4	0.5	0.2
2	12.5	3.0	0.9	0.5	0.3
3	8.0	4.0	2.1	0.4	0.3
4	13.8	5.1	1.8	0.3	0.1

Comment: Most of the injected liposomal DHEA is removed from the circulation within the first 2 minutes.

Table 2: Tissue levels of liposomal DHEA 24 hours after i.v. injection.

Mouse	% of injected liposomal DHEA in total tissue			
	Liver	Spleen	Kidney	Lungs
1	0.6	< 0.1	0.1	< 0.1
2	0.4	< 0.1	0.2	< 0.1
3	0.3	< 0.1	0.1	< 0.1
4	0.5	< 0.1	0.0	< 0.1

Comment: Very little liposomal DHEA recovered in tissues at 24 hours.

Study 2: Blood and tissue levels of liposomal DHEA soon after i.v. injection.

This study was undertaken to (a) confirm the rapid clearance of liposomal DHEA observed in Table 1 and (b) measure tissue levels soon after injection. Results are shown in Table 3.

Table 3: Blood and tissue levels of liposomal DHEA min after i.v. injection

Mouse	% of injected liposomal DHEA in total blood or tissue				
	Blood	Liver	Spleen	Kidney	Lungs
1	5.5	18.5	1.0	8.0	2.1
2	8.5	14.7	2.1	6.5	2.5
3	6.6	21.6	1.8	4.8	1.8
4	4.5	20.7	1.5	8.2	1.5

Comment: Blood contains again very little of the injected radioactivity 5 min after injection. Tissues contain more at 5 min than they did at 24 hrs. (see Table 2).

Study 3: Mouse survival after 5 intravenous injections of liposomal DHEA.

Five mice were injected i.v. once every day for 5 days with 0.9 mg liposomal DHEA. 24 hrs. after the last injection mice appeared healthy and remained so for at least two weeks.

Intravenous injection of liposomal DHEA (0.9 mg per mouse) led to the rapid removal of the drug from the circulation (nearly 90% removed within the first 2 min). This is in contrast with previous findings with liposomes of similar size (diameter less than 250 nm) where over 50% of the dose is recovered in blood circulation 2 min after injection. Such rapid clearance of DHEA liposomes suggests the possibility of the receptor for the drug, the latter probably being available on the liposomal surface. Examination of tissues revealed that the liver took up more of liposomal DHEA than spleen, kidneys and lungs. This is consistent with previous observations of liposomal fate. With regard to possible liposomal DHEA toxicity, this was not apparent in mice injected daily over five days.

The steroid dehydroepiandrosterone (DHEA) is solublised in a 45% solution of 2-hydroxy-propyl-beta-cyclodextrin (HPBCD) to a concentration of 47.8mg DHEA/ml of solution in water. This solution is then formulated into liposomes.

I CLAIM:

1. A liposomal drug delivery system wherein the hydrophobic component of the liposome is composed, in whole or in part,of a 17-ketosteroid.

2. A drug delivery system according to claim 1 wherein the 17-ketosteroid is dehydroepiandrosterone(DHEA).

3. A liposomal drug delivery system wherein a 17-ketosteroid is used to target the liposome to specific body tissue.

4. A drug delivery system according to claim 3 wherein the 17-ketosteroid is dehydroepiandrosterone (DHEA).

5. A drug delivery system according to claim 1,2,3,4 wherein the therapeutic agent is removed rapidly form circulation.

6. A liposomal drug delivery system wherein the hydrophilic component contains in whole or in part a 17-ketosteroids alond with one or more aliphatic carbocyclic compounds that have pendant hydroxy groups.

7. A drug delivery system according to claim 6 wherein the aliphatic carbocylic compounds that have pendant hydroxy groups is hydroxypropyl-beta-cyclodextrin (HPBCD).

8. A drug delivery system according to claim 6 wherein the 17-ketosteroid is dehydroepiandrosterone(DHEA).

9. A liposomal drug delivery system wherein the therapeutic agent is totally or partially a 17-ketosteroids.

10. A drug delivery system according to claim 9 wherein the 17-ketosteroid is dehydroepiandrosterone(DHEA).

11. A drug delivery system according to claim 10 wherein the therapeutic agent is complexed with one or more aliphatic carbocyclic compounds that have pendant hydroxy groups thus restricting the enzymatic transformation of DHEA to DHEA-sulphate within the body.

12. A liposomal drug delivery system wherein the hydrophilic and hydrophobic components contains in whole or in part a 17-ketosteroids along with one or more aliphatic carbocyclic compounds that have pendant hydroxy groups.

13. A drug delivery system according to claim 12 wherein the aliphatic carbocyclic compounds that have pendant hydroxy groups is hydroxypropyl-beta-cyclodextrin (HPBCD).

14. A drug delivery system according to claim 12 wherein the 17-ketosteroid is dehydroepiandrosterone(DHEA).

15. A liposomal drug delivery system wherein the hydrophilic component contains in whole or in part a complex of a therapeutic agent with one or more aliphatic carbocyclic compounds that have pendant hydroxy groups.

16. A drug delivery system according to claim 15 wherein the therapeutic agent is interleukin-2.

17. A drug delivery system according to claim 15 wherein the therapeutic agent is tumor necrosis factor.

18. A drug delivery system according to claim 15 wherein the therapeutic agent is insulin.

19. A drug delivery system according to claim 15 wherein the therapeutic agent is Alpha-fetoprotein or antibodies to parts thereof.

20. A drug delivery system according to claim 15 wherein the aliphatic carbocyclic compounds that have pendant hydroxy groups is hydroxypropyl-beta-cyclodextrin (HPBCD).

21. A drug delivery system according to claim 15 wherein the alphatic carbocyclic compounds that have pendant hydroxy groups is alpha-cyclodextrin.

22. A drug delivery system according to claim 15 wherein the aliphatic carbocyclic compounds that have pendant hydroxy groups is beta-cyclodextrin.

23. A drug delivery system according to claim 15 wherein the aliphatic carbocyclic compounds that have pendant hydroxy groups is gamma-cyclodextrin.

Patrick F. McCardle

ABSTRACT

The present invention relates to the use of aliphatic carbocyclic compounds that have pendant hydroxy groups for incorporating water-insoluble molecules as hydrophilic components of liposomal drug delivery systems for human or animal treatment.

In particular this patent describes a liposomal drug delivery system wherein the hydrophilic component contains in part a 17-ketosteroids along with aliphatic carbocyclic compounds that have pendant hydroxy groups, such as cyclodextrin (CD), and the likes, as well as mixtures thereof. In particular the 17-ketosteroids is Dehydroepiandrosterone(DHEA) and the cyclodextrin is hydroxypropyl-beta-cyclodextrin. The cyclodextrin is used with the 17-ketosteroid to give it hydrophilic characteristics.

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